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Antibacterial and Antifungal Studies of the Crude Extract and Solvent Fractions of *Onosma khyberianum*

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ABSTRACT

Background: The most commonly used plant namely *Onosma khyberianum* was brought under antimicrobial study after observing their medicinal values. The present study aim to assess antimicrobial activity of ethanolic extracts and subsequent solvent soluble fractions of *Onosma khyberianum* against five bacterial strains *Salmonella typhi*, *Shigella dysenteriae*, *Escherichia coli*, *Staphylococcus aureus*, *Vibrio cholerae* and three fungal strains *Aspergilus flavus*, *Alternaria alternate* and *Fusarium oxysporum*. **Materials and Methods:** Briefly the stock solution of crude extract and other fractions were prepared in DMSO (3 mg mL^{-1} for antibacterial essay, 4 mg 1 mL^{-1} for antifungal essay). The antibacterial activity was evaluated by agar diffusion method while for antifungal assay the disc diffusion method was used. **Results:** The ethanolic and chloroform fractions showed excellent activity against all the selected bacterial strains. The same fractions also showed best activity against all selected fungal strains. **Conclusion:** The present study suggests *Onosma khyberianum* to be a source for isolation of antimicrobial compounds for human health care and use as preservatives in food processing industries.

Key words: *Onosma khyberianum*, extraction, fractionation, antimicrobial activity

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INTRODUCTION

Medicinal plants are paying attention of researchers in recent years. In fact medicinal plants hold secondary metabolites as alkaloid, tannin, terpenoids, flavonoids etc., the existence of which is proved by *in vitro* study of these plants (Sher, 2009). The genus *Onosma* (Boraginaceae) consists of 85 species, occurring mainly in Iran and westwards to Syria, Turkey and Europe. *Onosma* is a genus of plants that grow biennially or perennially. It is represented in Pakistan by 08 species, namly *O. khyberianum*, *O. limitaneum*, *O. chitralicum*, *O. hispida*, *O. dichroantha*, *O. hypoleucum*, *O. griffithii* and *O. thomsonii* (Ali and Nasir, 1989). The plants of the genus has cooling, laxative, anthelminitic, alexipharmic effects and are effective in the diseases of the eye, derangements of blood, bronchitis, abdominal pain, stangury, thirst, itch, leucoderma, fever, wound, pile and in urinary calculi (Kirtikar, 1994). Most of the ailments like diarrhea, diabetes, malaria, skin diseases and microbial diseases are cured by plants in different ways like intake of fruits and vegetables, in form of drugs and extracts. The world health organization (WHO) reported that 80% of people favor to use medicinal plants to cure these diseases (Sahito *et al*., 2003). Since herbal drugs have compounds having antimicrobial properties so they act as antimicrobial agents (Prince and Prabakaran, 2011). Antimicrobial agents or antimicrobials are substances that kill or restrain the growth of other microorganisms such as bacteria, fungi or protozones. In early 20th century, antibiotics were considered only those drugs that were only formed by one microorganism that demolish or inhibit the growth of another microorganism but in current days antibiotics are referred to all those drugs that are used to cure microbial diseases. It means not only antibiotics but all synthetic compounds also come under the class of antimicrobial agents (Levy, 1994). Fungal diseases are not common as bacterial diseases but if occurred they are complicated to cure especially in those having weak immune system (Kivcak *et al*., 2009). After the discovery of antibiotics it was considered that infectious diseases would be entirely treated but with the enlarged use of antibiotics, the causal agents of the diseases i.e., bacteria and fungi modified their physiology and in this new form these are resistant to the pre-existing antibiotics. Moreover most of the antibiotics are toxic. That is why the search of a secure source of new antibiotics or antimicrobial agents are required to overcome this global therapeutic problem (Sibanda and Okoh, 2007).

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Botanist, ethnopharmacologist, natural products chemist and microbiologist are trying to uprooting new antimicrobial drugs from medicinal plants in order to cover the demand for natural and non costly drugs to cure infectious diseases without any side effects (Cowan, 1999). The unnecessary use of antibiotics for crop production and resistance of microorganism against these antibiotics has made various health problems to human being. Medicinal plants have compounds to control microorganisms, so it is significant to isolate those compounds and to know those strategies by which they operate and kill pathogenic bacteria (Kuntal *et al*., 2010). The process of evaluating antimicrobial compounds is therefore a continuing process.

Plant extracts have great potential as antimicrobial compounds against microorganisms. Thus, they can be used in the treatment of infectious diseases caused by resistant microbes. Surprisingly no antimicrobial studies have been made so far on *Onosma khyberianum*. The objective of this research was to evaluate the potential of *Onosma khyberianum* extract and solvents partitions on microbial strains.

MATERIALS AND METHOD

Collection of plant material: The *Onosma khyberianium* plant was collected from Khyber Agency which was used for the treatment of infection diseases by tribal peoples and was authenticated by plant taxonomist at Department of Botany (voucher No. 156), KUST, Kohat, Khyber Pakhtunkhwa, Pakistan.

Preparation of plant extract: The whole plant was shade dried and then chopped into fine powder. The fine powder of plant was soaked in ethanol for 15 days, extracted three times at room temperature in the same solvent and filtered. The filtrates were then processed through rotary evaporator to get crude extract and dried. The dried extract was further suspended in water and was partitioned successively with n-hexane, ethyl acetate, chloroform and n-butanol to obtain n-hexane, chloroform, ethyl acetate, n-butanol and aqueous soluble fractions, respectively. The crude extract and its solvent soluble fractions were tightly packed and stored in refrigerator at 4°C.

Antibacterial activity: The crude extract and their respective solvents soluble fractions were subjected to antibacterial evaluation against five bacterial stains i.e., *Salmonella typhi*, *shigella dysenteriae*, *Escherichia coli*, *Staphylococcus aureus* and *Vibrio cholerae*. Solutions of crude extract and various fractions at concentration of 3 mg mL^{-1} were prepared in DMSO.

The Nutrient agar media 14 g was prepared in 500 mL conical flask and it was sterilized along with Petri dishes, cork borer and pipette in autoclave for 15 min at 121°C at high pressure. The nutrient agar media was poured into Petri dishes under laminar flow hood to evade bacteria from environment. Wells of 7 mm were punched in the agar media by using sterile metallic borer. The modified agar diffusion method as reported by Khan and Tewari (2011), was followed. Nutrient agar media was inoculated with a given bacterial culture corresponding to 10^6 CFU mL^{-1}. Bacterial strains was spread on the solidified agar media. The prepared stock solutions of extract and fractions were poured to wells. The petri dishes were incubated at 37°C for 24 h and control wells containing antibiotic Chloramphenicol, which is a positive control, was also run side by side in the same petri dishes and DMSO was used as negative control. After 24 h antibacterial activities were measured by measuring the diameter of the zones of inhibition and were compared these values of zone of inhibitions with the zone of inhibition of standard drug levoflaxcine. The amount of growth in each well was measured (Khan and Tewari, 2011).

Antifungal assay: Three fungal strains i.e., *Aspergilus flavus*, *Alternaria alternate* and *Fusarium oxysporum* were used for the antifungal activity. For Antifungal assay disc diffusion method was used (Perez *et al*., 1990). Briefly the stock solution of crude and other fractions were prepared in DMSO i.e., 4 mg mL^{-1}. Nutrient broth was used for culturing fungal strains. The media was prepared according to manufacture specification and was transferred to plates at 50-60°C under laminar flow hood. These plates were inoculated with the test fungi and incubated at 27°C for growth. For the antifungal assays nutrient agar was prepared by dissolving and autoclaving the specified quantity of dry powder of Nutrient agar in a given quantity of distilled water and was transferred to plates. One milliliter solution of the test sample was added and inoculated with different fungal species. Plates were incubated at 27°C for 5 to 8 days and inhibition of fungal growth was measured (Perez *et al*., 1990).

RESULTS AND DISCUSSION

Among most critical health issues of the globe in 21st century, bacterial infection is considered to be one of them (Morris and Masterton, 2002). Bacterial defiance to antibiotics is the key health issue and thus, it is essential to overcome this problem by the development of new drugs with innovative mechanism of action (Wang *et al*., 2003). Plants herbal mixtures contributed a lot to human welfare and health, thus it provides a foundation for distinctive drug compounds. The utilization of plants various extracts with well known antimicrobial potential has significant value for therapeutic remedies.

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Table 1: Antibacterial Profile of *Onosma khyberianum*

	Zone of inhibition (mm)				
Fractions	*S. typhi*	*S. dysenteriae*	*E. coli*	*S. aureus*	*V. cholera*
Ethanol	7	21	5	11	20
n-hexane	0	8	0	9	12
Chloroform	28	26	11	11	26
Ethyl acetate	5	11	0	15	13
n-butanol	3	6	0	6	10
Aqueous	2	4	0	6	10
Positive control	39	40	39	39	38

Table 2: Antifungal profile of *Onosma khyberianum*

	Fungal strains used					
	Fusarium oxysporum		*Alternaria alternata*		*Aspergillus flavus*	
Extract/Fractions	Zone of inhibition (mm)	Inhibition (%)	Zone of inhibition (mm)	Inhibition (%)	Zone of inhibition (mm)	Inhibition (%)
Ethanol	18	51.4	13	35.1	7	18.9
n-hexane	0	0.0	7	18.9	0	0.0
Chloroform	17	48.6	11	29.7	9	24.3
Ethyl acetate	8	22.9	0	0.0	0	0.0
n-butanol	5	14.3	0	0.0	0	0.0
Aqueous	0	0.0	0	0.0	0	0.0
Positive control	35	100.0	37	100.0	37	100.0

In our present exploration, the antibacterial and antifungal activity of *Onasma khyberianum* various extract/fractions in comparison with Chloramphenicol and Terbinafine standards were determined against five bacterial *Salmonella typhi*, *Shigella dysenteriae*, *Escherichia coli*, *Staphylococcus aureus*, *Vibrio cholera* and three fungal strains *Alternaria alterrnata, Aspergillus flavus* and *Fusarium oxysporum,* respectively.

The antibacterial activities of *Onasma khyberianum* have been shown by Table 1. None of the fractions were completely inactive against any bacterial strain. Results of antibacterial attempt revealed that chloroform and methanol fractions of plant displayed more potent activity against various strains as compared to n-hexane, n-butanol and aqueous fractions. Promising activity 28 mm was shown by chloroform fraction against *Salmonella typhi* followed by same fraction against *Shigella dysenteriae* and *Vibrio cholera* with inhibition zone of 26 mm for each. Ethanol fraction showed best activity against *Shigella dysenteriae* (21 mm) and *Vibrio cholera* (20 mm). n-hexane showed activity against *Shigella dysenteriae* (8 mm) and *Vibrio cholera* (12 mm) but completely ineffective against *Salmonella* and *E. coli*. All fractions showed excellent activity against *shigella, Stap. aureus and Vibrio cholera.*

The antifungal activities of *Onasma khyberianum* against three fungal strains *Alternaria alterrnata, Aspergillus flavus* and *Fusarium oxysporum* have been presented in Table 2 from which it is clear that ethanol and chloroform fractions of plant were most active against all the selected fungal strains as compared to n-hexane, ethyl acetate, n-butanol and water. The activity of ethanol fraction against *F. oxysporum* was 18 mm, *A. alternata* 13 mm and *A. flavus* was 7 mm whereas the activities of chloroform fraction against *F. oxysporum, A. alternata* and *A. flavus* were 17, 11 and 9 mm, respectively. The activity of n-hexane recorded against *A. alternata* was 7 mm but it was entirely inactive against *F. oxysporum* and *A. flavus*. Similarly ethyl acetate and n-butanol fractions were only active against *F. oxysporum* and inactive for all other fungal strains while water fraction showed no activity against none of the fungal strain.

Ozgen *et al*. (2003) studied the antibacterial activity of two species of genus *onosma*. The ethyl acetate fraction was found to be effective on *Staphyloccoccus aureus*, *Bacillus subtilis* and *Escherichia coli*. The chloroform fractions was the most effective on *S. aureus* and *E. coli*, respectively. These two species did not have any antifungal activity against any fungal strain. Here ethyl acetate fraction donot show any promising activity against any of the bacterial strains. The chloroform fraction show excellent activity against *S. aureus*, *S. dysenteriae* and *V.cholerae* (Ozgen *et al*., 2003). In antifungal study crude ethanolic extract show good results against *Fusarium oxysporum* and *Alternaria alternate*. In fractions chloroform fraction show good activity against *Fusarium oxysporum* (Ozgen *et al*., 2003).

Naz *et al*. (2006) reported antibacterial activity of *Onosma hispidum* for the first time in this species. In addition to these compounds, the crude ethanolic extract and methanol fraction exhibited substantial bioactivity against species of corynebacteria, enterococci, staphylococci and streptococci (Naz *et al*., 2006).

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CONCLUSION

These findings suggest that *Onosma khyberianum* has good antibacterial and antifungal properties that can be used for infection control and treatment and could also be as new source for antibiotics discovery and infection treatment. Further studies are necessary to isolate and characterize the active components of the extracts/fractions and also to elucidate their antibacterial mechanisms of action.

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